REMARKS

Applicants acknowledge, with appreciation, grant of their earlier request for continued examination and withdrawal of the finality of the previous Office Action under 37 C.F.R. § 1.114.

In order to expedite allowance of this application, applicants have cancelled claims 5, 17, 54-79 and 81-88. These claim cancellations are not to be interpreted as applicants' acquiescence to any of the outstanding rejections and are made without prejudice to applicants' right to pursue the subject matter of the cancelled claims in one or more applications claiming priority herefrom under 35 U.S.C. § 120. Applicants have also amended the claims to correct various typographical errors. Finally, applicants have amended claim 7 to recite wherein said "change in chemical composition of the environment of surrounding said crystal" while deleting the phrase "dilution of said protein crystal in a solution." Support for this amendment can be found on page 60, lines 3-28 to page 61, lines 1-5 of the specification. None of these amendments constitutes new matter.

Applicants request reconsideration of the above-identified application in view of the foregoing amendments and the following remarks.

35 U.S.C. § 132 - New Matter

Applicants' October 23, 2001 amendment to page 69 of the specification stands objected to on the basis that it purportedly introduces new matter by reciting crosslinked protein crystals that are between about 30% and about 80% as soluble as an uncrosslinked counterpart of said protein crystal when stored in phosphate buffered saline solution at about 30° C for between about 100 hours and about 350 hours. Without conceding to this assertion, applicants have cancelled the objected-to text from the specification.

35 U.S.C. § 112, First Paragraph - Lack of Support

Claims 1-79 and 81-88 stand rejected under 35 U.S.C. § 112, first paragraph, as not containing adequate support for "insertion a) in claims 1, 17, 18 and 54 and insertion b) in claims 55 and 56 that recites 'between about 30% and about 80% as soluble as an uncrosslinked counterpart of said protein crystal when stored in phosphate buffered saline solution at about 30° C for between about 100 hours and about 350 hours'." In addition, the Examiner asserts that "adequate support is not found in the specification for reciting 'releasing between about 0.1% and about 100% of crystalline protein as soluble protein per day' in b) of claims 1, 17, 18 and 54, c) of claim 55 and a)

of claim 56." Applicants point to page 10, lines 27-31 of the specification to support this recitation. The Examiner further asserts that "adequate support is not found in the specification for reciting in claim 19 'the amino acid residues involved in the crosslinks, whether the crosslinker is homobifunctional or heterobifunctional'." Applicants point to page 26, lines 30-31 of the specification to support this recitation. The Examiner also asserts that "adequate support is not found in the specification for reciting in claim 86 'releasing about 100% of crystalline protein as soluble protein per day', in claim 87 'releasing about 100% of crystalline protein as soluble protein per hour' and in claim 88 'releasing between about 1% and about 50% of crystalline protein as soluble protein per minute'." Again, applicants point to page 10, lines 27-34 to page 11, lines 1-2 of the specification to support these recitations. Without conceding to the merits of these objections, applicants have cancelled the objected-to recitations from the amended claims.

35 U.S.C. § 112, Second Paragraph - Indefiniteness

Claims 1-79 and 81-88 stand rejected under 35 U.S.C. § 112, first paragraph, as "being indefinite for failing to particularly point out and distinctly claim the subject matter

which applicant regards as the invention." More particularly, the Examiner contends that "the specification fails to support that any environment change within the scope of environment changes claimed will provide the claimed solubility." This contention is believed to be moot in view of the claim amendments set forth herein. Specifically, the claims have been amended to delete specific levels of solubility added in response to the prior Office Action. Applicants believe that the data in the specification fully supports the environment changes recited in the amended claims.

The Examiner also objected to various phrases within the wording of claims 54-56 and 67-75. These objections are obviated by applicants' cancellation of those claims in this application. As stated above, those claim cancellations are directed toward expediting allowance of this application and do not represent applicants' concession to the merits thereof.

35 U.S.C. § 102(a) - Anticipation

Claims 1-44, 46-63, 76 and 81-88 stand rejected under 35 U.S.C. § 102(a) as being "anticipated by" Navia et al. (United States patent 5,618,710) ("Navia"). Specifically, the Examiner contends that "Navia et al disclose crosslinked protein crystals that will inherently be capable of being changed to

soluble form as claimed by one or more of the claimed environment changes since the crosslinked protein crystals disclosed by Navia et al can be prepared using crosslinking conditions that will result in essentially the same or less crosslinking than obtained when using crosslinking conditions disclosed in the present specification." The Examiner further cites the crosslinker concentrations used in Examples 2, 4-7 and 9-10 of Navia and Examples 18-20, 22 and 23 of the present application; asserting that "the crosslinking conditions used by Navia et al are essentially equivalent to those disclosed by the present specification and would not have resulted in a substantially greater amount of crosslinking." Applicants traverse.

Applicants maintain their previous argument that crosslinked protein crystals according to Navia are not inherently capable of change from insoluble to soluble form when subjected to the same change in environment that causes dissolution of the crosslinked protein crystals according to the present application. In particular, Navia is directed to crosslinked protein crystals wherein the resulting crystals can "function at elevated temperatures, extremes of pH, and in harsh aqueous, organic, or near-anhydrous media" (column 3, lines 49-51). In contrast, the crosslinked crystals of the present

application dissolve when subjected to a change in environment such as pH, temperature, organic solvents, chemical composition and solute concentration. This lack of inherency for such controlled dissolution crystals based on Navia is demonstrated in the Declaration of Bhami C. Shenoy, Ph.D, ("Shenoy Declaration"), filed concurrently herewith.*

The Shenoy Declaration sets forth side-by-side "dissolution" results for crosslinked protein crystals according to Navia, as compared with controlled dissolution crosslinked protein crystals according to the present application.

As detailed in the Shenoy Declaration, crosslinked protein crystals according to Navia (2.0% and 7.5% glutaraldehyde concentration) were prepared and their "dissolution" properties compared to those of crosslinked protein crystals according to Examples 18-23 of the present application (for crystals crosslinked with 4%, 6.5% or 6.0% glutaraldehyde concentration). In addition, the "dissolution" property of PeptiCLEC™-TR (Altus), a commerically-available crosslinked crystalline thermolysin, was also compared to the crosslinked protein crystals of the present application. The

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*The enclosed facsimile copy of the executed version of the Shenoy Declaration will be followed shortly by the original.

results demonstrate that Navia's crosslinked protein crystals were insoluble, or essentially insoluble, when placed in the same dissolution conditions as those set forth in Examples 24 and 25 of the present application. In contrast, the crosslinked protein crystals prepared according to Examples 18-23 of the present application exhibited increasing dissolution over a 3 hr to 24 hr period.

Based on these results, it cannot be said that crosslinked protein crystals according to Navia are inherently capable of change from insoluble to soluble form when subjected to the same change in environment that causes dissolution of the crosslinked protein crystals according to the present application. Accordingly, the claim rejections under 35 U.S.C. § 102(a) should be withdrawn.

35 U.S.C. § 103(a) - Obviousness

Claims 45 and 64-66 stand rejected under 35 U.S.C. § 103(a) as "being unpatentable over Navia et al." Specifically, the Examiner asserts that "it would have been obvious to use a crosslinked enzyme crystal such as protease produced as disclosed by Navia et al in a detergent formulation..." and "it would have been a matter of obvious

choice to use known crosslinking agents other than those disclosed by Navia et al." Applicants traverse.

As demonstrated in the Shenoy Declaration, the crosslinked protein crystals of Navia are not the controlled dissolution crystals of the present invention. Accordingly, Navia does not obviate the use of controlled dissolution crosslinked protein crystals according to the present invention in detergent formulations. Nor would it be obvious to use crosslinking agents other than those disclosed by Navia to provide controlled dissolution protein crystals according to the present invention."

Claims 1-79 and 81-88 stand rejected under 35 U.S.C. § 103(a) as being "unpatentable over Navia et al in view of" United States patent 5,066,490 ("Neville") and United States patent 5,508,164 ("Kausch"). Specifically, the Examiner asserts that it "would have been obvious to use a reversible crosslinking agent such as a disulfide agent as the crosslinking agent of Navia et al to obtain reversible immobilization as suggested by Neville and Kausch." Applicants traverse.

As discussed above, crosslinked protein crystals according to Navia are not inherently capable of change from insoluble to soluble form when subjected to the same change in environment that causes dissolution of crosslinked protein

crystals according to the present application. Instead, the Navia crystals are designed to withstand elevated temperatures, extremes of pH and in harsh aqueous, organic, or near-anhydrous media. Given the stability characteristics of Navia's crosslinked protein crystals, there would be no motivation for one of skill in the art to replace the crosslinking agents disclosed in Navia with the reversible crosslinkers of Neville or Kausch.

Non-Statutory Double Patenting

Claims 1-76 and 81-88 stand rejected under the judicially created doctrine of obviousness-type double patenting as being "unpatentable over" claims 1-19 of United States patent 6,140,475. The Examiner asserts that "although the conflicting claims are not identical, they are not patentably distinct from each other because crosslinked protein crystals that dissolve as presently claimed and [the] method for preparation thereof as presently claimed would have been obvious from the method of the claims of the patent that produces crosslinked protein crystals that dissolve as a result of a change in environment that can be the same as required by the present claims." To the extent that the foregoing claim amendments have not rendered this rejection moot, applicants

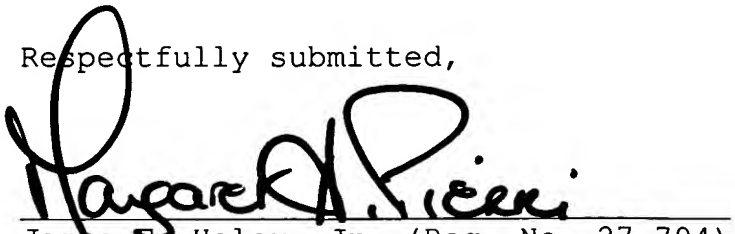
stand ready to file a Terminal Disclaimer, if appropriate, upon the Examiner's indication of allowable subject matter in this application.

Claims 77-79 also stand rejected for obviousness-type double patenting as being "unpatentable over" claims 1-19 of United States patent 6,140,475, in view of Neville (United States patent 5,066,490) and Kausch (United States patent 5,508,164). This rejection is rendered moot in view of applicants' cancellation of claims 77-79.

CONCLUSION

Applicants request that the Examiner consider the foregoing amendments and remarks and pass this application to issue.

Respectfully submitted,



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Appendix A

We Claim:

C1

1. (Three times amended) A crosslinked protein crystal, said protein crystal being crosslinked with a multifunctional crosslinking agent such that said protein crystal is capable of controlled dissolution from insoluble and stable form to soluble and active form upon a change in the environment surrounding said crystal, said change being selected from the group consisting of: change in temperature, change in pH, change in chemical composition, change from concentrate to dilute form and combinations thereof.

C2

2. (Twice amended) The crosslinked protein crystal according to claim 1, wherein said change from concentrate to dilute form comprises dilution of said protein crystal in a solution.

3. (Not presently amended) The crosslinked protein crystal according to claim 2, wherein said dilution of said protein crystal in a solution comprises an increase or decrease in salt concentration.

4. (Not presently amended) The crosslinked protein crystal according to claim 3, wherein said dilution of said protein crystal in a solution comprises a decrease in salt concentration.

5. (Cancelled)

6. (Twice amended) The crosslinked protein crystal according to claim 2, wherein said dilution of said protein crystal in a solution comprises an increase in water concentration.

C3

7. (Twice amended) The crosslinked protein crystal according to claim 1, wherein said change in chemical composition of the environment surrounding said crystal comprises an increase in organic solvent concentration.

8. (Not presently amended) The crosslinked protein crystal according to claim 2, wherein said dilution of said protein crystal in a solution comprises a decrease in detergent concentration.

9. (Not presently amended) The crosslinked protein crystal according to claim 2, wherein said dilution of said protein crystal in a solution comprises a decrease in protein conentration.

C4

10. (Amended) The crosslinked protein crystal according to claim 1, wherein said change from concentrate to dilute form comprises a change in concentration of all solutes from about 2-fold to about 10,000-fold.

11. (Not presently amended) The crosslinked protein crystal according to claim 10, wherein said change from concentrate to dilute form comprises a change in concentration of all solutes from about 2-fold to about 700-fold.

C5

12. (Twice amended) The crosslinked protein crystal according to claim 1 or 18, wherein said change in pH comprises a change from acidic pH to basic pH.

C6

13. (Amended) The crosslinked protein crystal according to claim 1 or 18, wherein said change in pH comprises a change from basic pH to acidic pH.

C7

14. (Twice amended) The crosslinked protein crystal according to claim 1 or 18, wherein said change in temperature comprises an increase or decrease in temperature.

15. (Not presently amended) The crosslinked protein crystal according to claim 14, wherein said change in temperature is an increase in temperature from a low temperature between about 0°C and about 20°C to a high temperature between about 25°C and about 70°C.

C8

16. (Twice amended) The crosslinked protein crystal according to claim 1, wherein said active form of said protein is a form which is active against macromolecular substrates.

17. (Cancelled)

C9

18. (Three times amended) A crosslinked protein crystal, said protein crystal being crosslinked by a multifunctional crosslinking agent such that said protein crystal is capable of releasing its protein activity at a controlled rate upon exposure to a change in the environment surrounding said crystal, said change being selected from the group consisting of: change in pH, change in solute

C9

concentration, change in temperature, change in chemical composition and combinations thereof.

C10

19. (Twice amended) The crosslinked protein crystal according to claim 18, wherein said controlled rate of releasing protein activity is determined by a factor selected from the group consisting of: the degree of crosslinking of said crosslinked protein crystal, the length of time of exposure of protein crystal to the crosslinker, the rate of addition of the crosslinking agent to said protein crystal, the nature of the crosslinker, the chain length of the crosslinker, the surface area of said crosslinked protein crystal, the size of said crosslinked protein crystal, the shape of said crosslinked protein crystal and combinations thereof.

20. (Not presently amended) The crosslinked protein crystal according to claim 18, wherein said crystal has a protein activity release rate between about 0.1% per day and about 100% per day.

21. (Not presently amended) The crosslinked protein crystal according to claim 18, wherein said crystal has a protein activity release rate between about 0.01% per hour and about 100% per hour.

22. (Not presently amended) The crosslinked protein crystal according to claim 18, wherein said crystal has a protein activity release rate between about 1% per minute and about 50% per minute.

C11

23. (Twice amended) The crosslinked protein crystal according to claim 1 or 18, said protein crystal being substantially insoluble and stable in a composition

C11 under storage conditions and substantially soluble and active under conditions of use of said composition.

24. (Not presently amended) The crosslinked protein crystal according to claim 23, wherein said composition is selected from the group consisting of cleaning agents, detergents, personal care compositions, cosmetics, pharmaceuticals, veterinary compounds, vaccines, foods, feeds, diagnostics and formulations for decontamination.

25. (Not presently amended) The crosslinked protein crystal according to claim 24, wherein said detergent is selected from the group consisting of powdered detergents, liquid detergents, bleaches, household cleaners, hard surface cleaners, industrial cleaners, carpet shampoos and upholstery shampoos.

C12 26. (Amended) The crosslinked protein crystal according to claim 24, wherein said cosmetic is selected from the group consisting of creams, emulsions, lotions, foams, washes, gels, compacts, mousses, slurries, powders, sprays, foams, pastes, ointments, salves, balms, shampoos, sunscreens and drops.

C13 27. (Twice amended) The crosslinked protein crystal according to claim 1 or 18, wherein said protein is an enzyme.

28. (Not presently amended) The crosslinked protein crystal according to claim 27, wherein said enzyme is selected from the group consisting of hydrolases, isomerases, lyases, ligases, transferases and oxidoreductases.

29. (Not presently amended) The crosslinked protein crystal according to claim 28, wherein said enzyme is selected from the group consisting of proteases, amylases, cellulases, lipases and oxidases.

C14

30. (Twice amended) The crosslinked protein crystal according to claim 1 or 18, wherein said protein is selected from the group consisting of therapeutic proteins, cleaning agent proteins, personal care proteins, veterinary proteins, food proteins, feed proteins, diagnostic proteins and decontamination proteins.

C15

31. (Three times amended) The crosslinked protein crystal according to claim 1 or 18, wherein said protein is selected from the group consisting of hormones, antibodies, inhibitors, growth factors, trophic factors, cytokines, lymphokines, growth hormones, nerve growth hormones, bone morphogenic proteins, toxoids and nutrients.

C16

32. (Twice amended) The crosslinked protein crystal according to claim 1 or 18, wherein said protein is selected from the group consisting of insulin, amylin, erythropoietin, Factor VIII, TPA, dornase-α, α-1-antitrypsin, urease, fertility hormones, FSH, LSH, postridical hormones, tetanus toxoid and diptheria toxoid.

33. (Twice amended) The crosslinked protein crystal according to claim 1 or 18, said crystal having a longest dimension of between about 0.01 μm and about 500 μm.

34. (Twice amended) The crosslinked protein crystal according to claim 1 or 18, said crystal having a longest dimension of between about 0.1 μm and about 50 μm.

35. (Twice amended) The crosslinked protein crystal according to claim 1 or 18, said crystal having a shape selected from the group consisting of: spheres, needles, rods, plates, rhomboids, cubes, bipyramids and prisms.

C16

36. (Twice amended) A composition comprising a crosslinked protein crystal according to claim 1 or 18, said composition being selected from the group consisting of cleaning agents, detergents, personal care compositions, cosmetics, pharmaceuticals, veterinary compounds, vaccines, foods, feeds, diagnostics and formulations for decontamination.

37. (Not presently amended) The composition according to claim 36, wherein said detergent is selected from the group consisting of powdered detergents, liquid detergents, bleaches, household cleaners, hard surface cleaners, industrial cleaners, carpet shampoos and upholstery shampoos.

C17

38. (Amended) The composition according to claim 36, wherein said cosmetic is selected from the group consisting of creams, emulsions, lotions, foams, washes, gels, compacts, mousses, slurries, powders, sprays, foams, pastes, ointments, salves, balms, shampoos, sunscreens and drops.

C18

39. (Twice amended) A protein delivery system, said system comprising crosslinked protein crystals according to claim 1 or 18, and a delivery device.

40. (Not presently amended) The protein delivery system according to claim 39, wherein said protein is

selected from the group consisting of: detergent enzymes, cosmetic proteins, pharmaceutical proteins, agricultural proteins, vaccine proteins and decontamination proteins.

41. (Not presently amended) The protein delivery system according to claim 40, said protein delivery system being a microparticulate protein delivery system.

42. (Not presently amended) The protein delivery system according to claim 41, wherein said microparticulate protein delivery system comprises crosslinked protein crystals having a longest dimension of between about 0.01 μm and about 500 μm.

43. (Not presently amended) The protein delivery system according to claim 42, wherein said microparticulate protein delivery system comprises crosslinked protein crystals having a longest dimension of between about 0.1 μm and about 50 μm.

C19

44. (Amended) The protein delivery system according to claim 41, wherein said microparticulate protein delivery system comprises crosslinked protein crystals having a shape selected from the group consisting of: spheres, needles, rods, plates, rhomboids, cubes, bipyramids and prisms.

C20

45. (Twice amended) A detergent formulation comprising a crosslinked protein crystal according to claim 1 or 18.

46. (Twice amended) A controlled release formulation comprising a crosslinked protein crystal according to claim 1 or 18.

C20

47. (Twice amended) A pharmaceutical controlled release formulation comprising a crosslinked protein crystal according to claim 1 or 18.

48. (Not presently amended) A pharmaceutical controlled release formulation comprising a crosslinked protein crystal, said protein crystal being crosslinked by a multifunctional crosslinking agent and said crystal being substantially insoluble under storage conditions and capable of releasing its protein activity *in vivo* at a controlled rate.

49. (Not presently amended) The pharmaceutical controlled release formulation according to claim 47, said pharmaceutical being capable of administration by parenteral or non-parenteral routes.

50. (Not presently amended) The pharmaceutical controlled release formulation according to claim 49, said pharmaceutical being capable of administration by oral, pulmonary, nasal, aural, anal, dermal, ocular, intravenous, intramuscular, intraarterial, intraperitoneal, mucosal, sublingual, subcutaneous or intracranial route.

51. (Not presently amended) The pharmaceutical controlled release formulation according to claim 47, wherein said pharmaceutical is capable of administration by oral route and said crosslinked protein crystal is substantially insoluble under gastric pH conditions and substantially soluble under small intestine pH conditions.

C21

52. (Twice amended) A vaccine comprising a crosslinked protein crystal according to claim 1 or 18.

C21

53. (Twice amended) A formulation comprising a crosslinked protein crystal according to claim 1 or 18, said formulation being selected from the group consisting of tablets, liposomes, granules, spheres, microspheres, microparticles and capsules.

Claims 54-79 and 81-88 (Cancelled)

89. (Added) The crosslinked protein crystal according to claim 1 or 18, wherein said multifunctional crosslinking agent is a homobifunctional crosslinker or a heterobifunctional crosslinker.

Crosslinker	Crosslinker Concentration	Crosslinking Time
GA	1.0%	1.5h
GA	0.25%	2h
GA	0.2%	2h
GA	0.15%	2h
NP/GA	0.1%/0.1%	5h/1.5h
411/GA	0.015%/0.035%	16h/1h
OA	0.2%	16h
OA	0.1%	16h
OA	0.05%	16h

The solubility profiles of the samples, shown in Figures 8 and 9, illustrate different rates of dissolution for the crosslinked crystals.

C1

Example 14 - Reversible Crosslinkers - Disulfide Crosslinked Subtilisin Crystals

We prepared subtilisin crystals (30-40 μm average, 27 mg/ml in Na_2SO_4) as previously described for subtilisin crystallization.

We then crosslinked the crystals using one of the following crosslinkers:

1) Dimethyl 3, 3'-dithiobispropionimidate•HCl - (DTBP) (Pierce)
2) Dithiobis(succinimidylpropionate) - (DSP)(Pierce)
3) 3, 3'- Dithiobis (sulfosuccinimidylpropionate) - (DTSSP) (Pierce).

Crosslinking was carried out in 15 ml neoprene screw cap tubes by placing 740 μl of subtilisin crystal slurry (20 mg) in 9.26 ml of buffer (25 mM $NaCO_3$/50 mM $NaHCO_3$, pH 8.0). One crosslinker was

Appendix B

We Claim:

1. (Three times amended) A crosslinked protein crystal, said protein crystal being crosslinked with a multifunctional crosslinking agent such that[:

a) said protein crystal is between about 30% and about 80% as soluble as an uncrosslinked counterpart of said protein crystal when stored in phosphate buffered saline solution at about 30°C for between about 100 and about 350 hours; and

b)] said protein crystal is capable of controlled dissolution from insoluble and stable form to soluble and active form [and releasing between about 0.1% and about 100% of crystalline protein as soluble protein per day] upon a change in the environment surrounding said crystal, said change being selected from the group consisting of: change in temperature, change in pH, change in chemical composition, change from concentrate to dilute form[, change in shear force acting upon the crystal] and combinations thereof.

2. (Twice amended) The crosslinked protein crystal according to [any one of] claim[s] 1, [or 86-88,] wherein said change from concentrate to dilute form comprises dilution of said protein crystal in a solution.

5. (Cancelled)

6. (Twice amended) The crosslinked protein crystal according to claim [5] 2, wherein said dilution of

said protein crystal in a solution comprises an increase in water concentration.

7. (Twice amended) The crosslinked protein crystal according to claim [2] 1, wherein said [dilution of said protein crystal in a solution] change in chemical composition of the environment surrounding said crystal comprises an increase [or decrease] in organic solvent concentration.

10. (Amended) The crosslinked protein crystal according to [any one of] claim[s] 1, [or 86-88,] wherein said change from concentrate to dilute form comprises a change in concentration of all solutes from about 2-fold to about 10,000-fold.

12. (Twice amended) The crosslinked protein crystal according to [any one of] claim[s] 1[,] or 18 [86-88], wherein said change in pH comprises a change from acidic pH to basic pH.

13. (Amended) The crosslinked protein crystal according to claim 1 or 18, wherein said change in pH comprises a change from basic pH to acidic pH.

14. (Twice amended) The crosslinked protein crystal according to [any one of] claim[s] 1[,] or 18 [86-88], wherein said change in temperature comprises an increase or decrease in temperature.

16. (Twice amended) The crosslinked protein crystal according to [any one of] claim[s] 1, [or 86-88] wherein said active form of said protein is a form which is active against macromolecular substrates.

17. (Cancelled)

18. (Three times amended) A crosslinked protein crystal, said protein crystal being crosslinked by a multifunctional crosslinking agent such that[:

a) said protein crystal is between about 30% and about 80% as soluble as an uncrosslinked counterpart of said protein crystal when stored in phosphate buffered saline solution at 30°C for between about 100 and about 350 hours; and

b)] said protein crystal is capable of releasing its protein activity at a controlled rate [of between about 0.1% and about 100% of crystalline protein as soluble protein per day] upon exposure to a change in the environment surrounding said crystal, said change being selected from the group consisting of: change in pH, change in solute concentration, change in temperature, change in chemical composition[, change in shear force acting upon the crystal] and combinations thereof.

19. (Twice amended) The crosslinked protein crystal according to claim 18, wherein said controlled rate of releasing protein activity is determined by a factor selected from the group consisting of: the degree of crosslinking of said crosslinked protein crystal, the length of time of exposure of protein crystal to the crosslinker,[the amino acid residues involved in the crosslinks, whether the crosslinker is homobifunctional or heterobifunctional] the rate of addition of the crosslinking agent to said protein crystal, the nature of the crosslinker, the chain length of the crosslinker, the surface area of said crosslinked protein crystal, the size of said crosslinked protein crystal, the shape of said crosslinked protein crystal and combinations thereof.

23. (Twice amended) The crosslinked protein crystal according to [any one of] claim[s] 1[, 17,] or 18 [or 86-88], said protein crystal being substantially insoluble and stable in a composition under storage conditions and substantially soluble and active under conditions of use of said composition.

26. (Amended) The crosslinked protein crystal according to claim 24, wherein said cosmetic is selected from the group consisting of creams, emulsions, lotions, foams, washes, gels, compacts, mousses, [sunscreens,] slurries, powders, sprays, foams, pastes, ointments, salves, balms, shampoos, sunscreens and drops.

27. (Twice amended) The crosslinked protein crystal according to [any one of] claim[s] 1[, 17] or 18, [or 86-88,] wherein said protein is an enzyme.

30. (Twice amended) The crosslinked protein crystal according to [any one of] claim[s] 1[, 17] or 18, [or 86-88,] wherein said protein is selected from the group consisting of therapeutic proteins, cleaning agent proteins, personal care proteins, veterinary proteins, food proteins, feed proteins, diagnostic proteins and decontamination proteins.

31. (Three times amended) The crosslinked protein crystal according to [any one of] claim[s] 1[, 17] or 18, [or 86-88,] wherein said protein is selected from the group consisting of hormones, antibodies, inhibitors, growth factors, trophic factors, cytokines, lymphokines, growth hormones, nerve growth hormones, bone morphogenic proteins, toxoids and nutrients.

32. (Twice amended) The crosslinked protein crystal according to [any one of] claim[s] 1[, 17] or 18, [or 86-88,] wherein said protein is selected from the group consisting of insulin, amylin, erythropoietin, Factor VIII, TPA, dornase-α, [α-1-antitripsin] α-1-antitrypsin, urease, fertility hormones, FSH, LSH, postridical hormones, tetanus toxoid and diptheria toxoid.

33. (Twice amended) The crosslinked protein crystal according to [any one of] claim[s] 1[, 17] or 18, [or 86-88,] said crystal having a longest dimension of between about 0.01 μm and about 500 μm.

34. (Twice amended) The crosslinked protein crystal according to [any one of] claim[s] 1[, 17] or 18, [or 86-88,] said crystal having a longest dimension of between about 0.1 μm and about 50 μm.

35. (Twice amended) The crosslinked protein crystal according to [any one of] claim[s] 1[, 17] or 18, [or 86-88,] said crystal having a shape selected from the group consisting of: spheres, needles, rods, plates, rhomboids, cubes, [bipryamids] bipyramids and prisms.

36. (Twice amended) A composition comprising a crosslinked protein crystal according to [any one of] claim[s] 1[, 17] or 18, [or 86-88,] said composition being selected from the group consisting of cleaning agents, detergents, personal care compositions, cosmetics, pharmaceuticals, veterinary compounds, vaccines, foods, feeds, diagnostics and formulations for decontamination.

38. (Amended) The composition according to claim 36, wherein said cosmetic is selected from the group

consisting of creams, emulsions, lotions, foams, washes, gels, compacts, [suncreens] mousses, slurries, powders, sprays, foams, pastes, ointments, salves, balms, shampoos, sunscreens and drops.

39. (Twice amended) A protein delivery system, said system comprising crosslinked protein crystals according to [any one of] claim[s] 1[, 17] or 18, [or 86-88,] and a delivery device.

44. (Amended) The protein delivery system according to claim 41, wherein said microparticulate protein delivery system comprises crosslinked protein crystals having a shape selected from the group consisting of: spheres, needles, rods, plates, rhomboids, cubes, [bipryamids] bipyramids and prisms.

45. (Twice amended) A detergent formulation comprising a crosslinked protein crystal according to [any one of] claim[s] 1[, 17] or 18[, 86-88].

46. (Twice amended) A controlled release formulation comprising a crosslinked protein crystal according to [any one of] claim[s] 1[, 17] or 18[, 86-88].

47. (Twice amended) A pharmaceutical controlled release formulation comprising a crosslinked protein crystal according to [any one of] claim[s] 1[, 17] or 18[, 86-88].

52. (Twice amended) A vaccine comprising a crosslinked protein crystal according to [any one of] claim[s] 1[, 17] or 18[, 86-88].

53. (Twice amended) A formulation comprising a crosslinked protein crystal according to [any one of] claim[s] 1[, 17] or 18[, 86-88], said formulation being selected from the group consisting of tablets, liposomes, granules, spheres, microspheres, microparticles and capsules.

54. (Cancelled)

55. (Cancelled)

56. (Cancelled)

57. (Cancelled)

58. (Cancelled)

59. (Cancelled)

60. (Cancelled)

61. (Cancelled)

62. (Cancelled)

63. (Cancelled)

64. (Cancelled)

65. (Cancelled)

66. (Cancelled)

67. (Cancelled)

68. (Cancelled)

69. (Cancelled)

70. (Cancelled)

71. (Cancelled)

72. (Cancelled)

73. (Cancelled)

74. (Cancelled)

75. (Cancelled)

76. (Cancelled)

77. (Cancelled)

78. (Cancelled)

79. (Cancelled)

81. (Cancelled)

82. (Cancelled)

83. (Cancelled)

84. (Cancelled)

85. (Cancelled)

86. (Cancelled)

87. (Cancelled)

88. (Cancelled)

Crosslinker	Crosslinker Concentration	Crosslinking Time
GA	1.0%	1.5h
GA	0.25%	2h
GA	0.2%	2h
GA	0.15%	2h
NP/GA	0.1%/0.1%	5h/1.5h
411/GA	0.015%/0.035%	16h/1h
OA	0.2%	16h
OA	0.1%	16h
OA	0.05%	16h

The solubility profiles of the samples, shown in Figures 8 and 9, illustrate different rates of dissolution for the crosslinked crystals. [For example, as shown in Figure 8, at 30°C the crosslinked crystals are about 30% to about 80% as soluble as an uncrosslinked counterpart protein crystal when stored in phosphate buffered saline solution for between about 100 and about 350 hours.]

Example 14 - Reversible Crosslinkers - Disulfide Crosslinked Subtilisin Crystals

We prepared subtilisin crystals (30-40 µm average, 27 mg/ml in Na_2SO_4) as previously described for subtilisin crystallization.

We then crosslinked the crystals using one of the following crosslinkers:

1) Dimethyl 3, 3'-dithiobispropionimidate•HCl - (DTBP) (Pierce)
2) Dithiobis(succinimidylpropionate) - (DSP)(Pierce)
3) 3, 3'- Dithiobis (sulfosuccinimidylpropionate) - (DTSSP) (Pierce).

Crosslinking was carried out in 15 ml neoprene screw cap tubes by placing 740 µl of subtilisin crystal slurry (20 mg) in 9.26 ml of buffer (25 mM $NaCO_3$/50 mM $NaHCO_3$, pH 8.0). One crosslinker was